

## Characterization of inflorescence-predominant *chitinase* gene in *Metroxylon sagu* via differential display

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**Abstract** Chitinase is an enzyme that catalyzes the degradation of chitin, commonly induced upon the attack of pathogens and other stresses. A cDNA (*MsChi1*) was isolated from *Metroxylon sagu* and expressed predominantly in the inflorescence tissue of *M. sagu*, suggesting its role in developmental processes. The *chitinase* cDNA was detected and isolated via differential display and rapid amplification of cDNA ends (RACE). Primers specific to *M. sagu chitinase* were used as probes to amplify the 3'-end and 5'-end regions of *chitinase* cDNA. Transcript analysis showed that *chitinase* is expressed in inflorescence and meristem tissues but was not detected in the leaf tissue. Sequence analysis of amplified cDNA fragments of 3'-end and 5'-end regions indicated that the *chitinase* cDNA was successfully amplified. The *M. sagu chitinase* cDNA isolated was approximately 1,143 bp long and corresponds to 312 predicted amino acids. Alignments of nucleotide and amino acid have grouped this *chitinase* to family 19 class I *chitinase*.

**Keywords** Chitinase · *Metroxylon sagu* · RACE-PCR · Differential display · Sago palm · Inflorescence-dominant

### Introduction

*Metroxylon sagu* or locally known as sago palm, belongs to the *Lepidocaryoid* subfamily of *Arecaceae* (*Palmae*). It is a once-flowering (hapaxanthic), tillering or suckering

perennial that thrives in swamp areas. The trunk of the *M. sagu* is used to obtain starch for human consumption (Flach 1984) and it is an important plant contributing to the economy of Sarawak. The advantages of *M. sagu* cultivation are that it requires no fertilization, has few natural pests or diseases and can be grown in swamp areas where it is impossible for other crops to thrive (Abd-Aziz 2002).

Chitinases are proteins that catalyze the hydrolysis of  $\beta$ -1,4-linkages of *N*-acetyl-D-glucosamine polymer of chitin; a major component of the exoskeleton of insects, crustacean shells and cell wall of many fungi (Bishop et al. 2000; El-Sayed et al. 2000; Passarinho and de Vries 2002). Chitinases are present in many higher plant species, although higher plants themselves do not contain chitin, chitosan or chitin-like substrate (Boller et al. 1983; Hirano et al. 1988). Chitinases are often described as pathogenesis-related proteins because they are constitutively expressed at low levels and increase dramatically in response to fungal, bacteria or viral infections (Graham and Sticklen 1994; van Loon 1999). Chitinases also play a role in plant defense mechanism by damaging chitin structures of parasites (Bishop et al. 2000; Odjakova and Hadjiivanova 2001). Apart from that, *chitinase* can also be induced by stress or elicitors such as wounding, salicylic acid and ethylene (Graham and Sticklen 1994; Leon et al. 2001).

Plant *chitinases* are classified in to classes I–V depending on their sequences and primary structures (Collinge et al. 1993; Neuhaus et al. 1996). Several studies have revealed that some *chitinase* are expressed at higher levels in healthy floral and flower-predominant organs such as potato (Wemmer et al. 1994) and tomato (Harikrishna et al. 1996). The expression of *chitinase* in flowers have also been detected in *Arabidopsis thaliana* (Samac et al. 1990; Passarinho et al. 2001), petunia (Leung 1992), parsley (Ponath et al. 2000), rice (Takakura et al. 2000) and

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